ASTROCYTES, FROM BRAIN GLUE TO COMMUNICATION ELEMENTS: THE REVOLUTION CONTINUES

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Abstract | For decades, astrocytes have been considered to be non-excitable support cells of the brain. However, this view has changed radically during the past twenty years. The recent recognition that they are organized in separate territories and possess active properties — notably a competence for the regulated release of 'gliotransmitters', including glutamate — has enabled us to develop an understanding of previously unknown functions for astrocytes. Today, astrocytes are seen as local communication elements of the brain that can generate various regulatory signals and bridge structures (from neuronal to vascular) and networks that are otherwise disconnected from each other. Examples of their specific and essential roles in normal physiological processes have begun to accumulate, and the number of diseases known to involve defective astrocytes is increasing.

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Among glial cells of the CNS, oligodendrocytes and microglia have long been recognized as having unique, specialized functions: myelination and host defence, respectively. By contrast, the role of astrocytes has remained much more enigmatic. They were initially considered to be the 'brain glue', providing an inert scaffold necessary for neuronal distribution and interactions. As the field evolved, astrocytes came to be thought of as support cells, necessary to ensure optimal neuronal functioning. In the late 1980s, the recognition that they express voltage-gated channels and neurotransmitter receptors began to attract interest in astrocytes as potential participants in intercellular communication. However, it is only in the past few years that previously unrecognized and surprising functions have been revealed for astrocytes, including the control of synapse formation and function, adult neurogenesis and brain vascular tone. The pace and relevance of recent discoveries highlights how, for several decades, our vision of astrocytes had been static, and how many aspects of their function remain to be understood. Knowledge of astrocyte functions is increasingly perceived to be essential for both understanding brain function and its pathology, and facilitating the treatment of neurological and psychiatric diseases.

One reason that the active properties of astrocytes have remained in the dark for so long relates to the differences between the excitation mechanisms of these cells and those of neurons. Until recently, the electrical language of neurons was thought to be the only form of excitation in the brain. As astrocytes do not generate action potentials, they were considered to be non-excitable and, therefore, unable to communicate. The finding that astrocytes can be excited non-electrically has expanded our knowledge of the complexity of brain communication to an integrated network of both synaptic and non-synaptic routes.

In this review, we focus primarily on a field that has, in recent years, contributed to one of the most striking revolutions in our understanding of astrocytes — rapid intercellular communication. Although there has been intense research in other fields of astrocyte physiology and pathology, and on Schwann cells (PNS glial cells that work in a similar way to astrocytes in terms of their contribution to synaptic

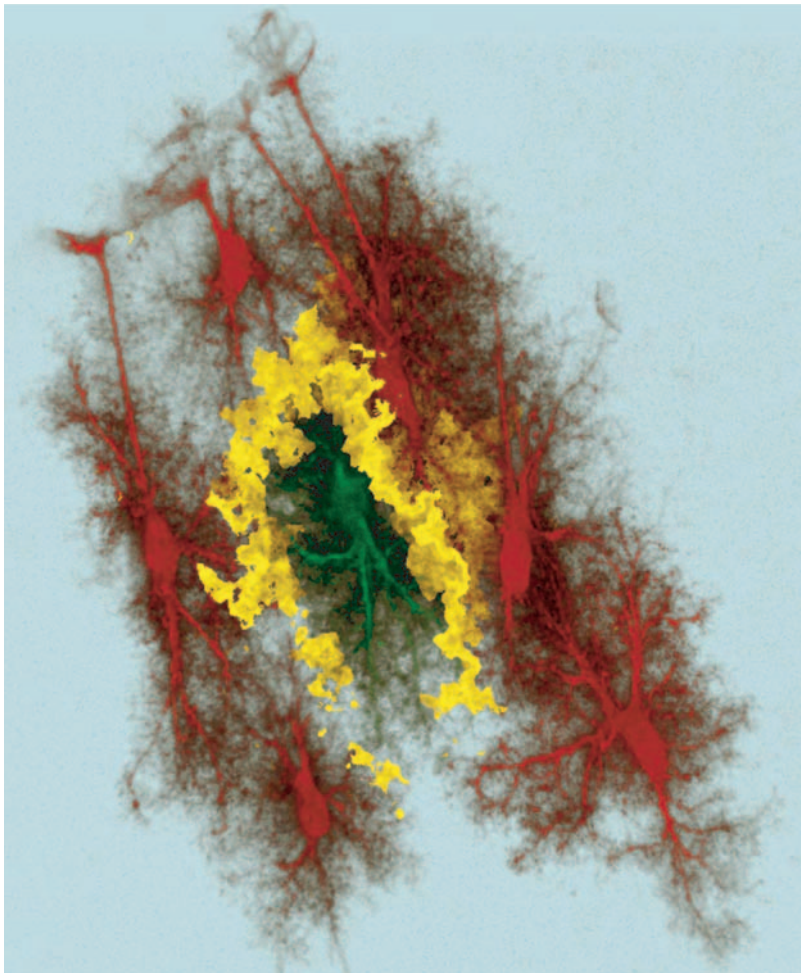


Figure 1 | **Three-dimensional view of the organization of astrocytes in separate anatomical domains.** The intermingling of protoplasmic astrocytes in the hippocampal CA1 molecular layer was examined by filling adjoining cells with different coloured fluorescent dyes (Alexa 468, a green fluorescent dye, and Alexa 488, a red fluorescent dye) by microinjection[3]. The discrete region of interaction of the fine terminal processes was revealed (yellow) by first blurring the images slightly (using a Gaussian blur filter) and then remapping the colour of the resultant area of overlap to bright yellow. This shows where the fine terminal processes of the adjoining astrocytes are closest to one another, although not actually overlapping. The 'boundary' of each astrocyte has a distinct surface that abuts neighbouring astrocytes. The long thin processes that extend from each cell shown in this figure are the 'siphon' processes of the astrocytes, which end in sheet-like surfaces that line the adjacent blood vessel. Image courtesy of E. Bushong and M. Ellisman, The National Center for Microscopy and Imaging Research, University of California, San Diego, USA.

functioning[1]), we focus instead on how astrocytes 'listen and talk to synapses'[2]. Here, we aim to define both the emerging modalities and the roles of this dialogue in the healthy and diseased brain. We also consider communication between astrocytes and other non-neuronal brain cells.

In view of the existence of an integrated communication network, we consider particularly informative studies performed *in vivo* and in semi-intact brain preparations, such as acute brain slices. By contrast, studies in primary cell cultures are considered with caution, and are therefore discussed most often as corroborative evidence to the results obtained using the more advanced preparations.

Astrocyte life in discrete territories

Advances in our understanding of astrocytes include new observations about their structure, organization and distribution. The architectural model of interdigitation between neighbouring astrocytes with overlapping processes — based on classic staining techniques and immunocytochemistry with the most widely used marker, glial fibrillary acidic protein (GFAP) — has been challenged by recent results obtained by microinjecting single hippocampal astrocytes with fluorescent dyes. This approach has revealed an ordered arrangement of these cells with minimal overlap (FIG. 1). Such an arrangement, which is established in the early postnatal period in parallel with neuronal and vascular territories, allows each astrocyte to cover a specific territory that interfaces with the microvasculature and that might include thousands of synapses[3,4]. However, fractions of this territory can be controlled autonomously by specialized astrocyte microdomains. These microdomains include lamellipodia and filopodia — fine expansions with unique structural composition[5] and motility that allow highly dynamic interactions with the surrounding synapses[6,7] — and the end-feet of the glial–vascular interface, from which signals can propagate to the next end-feet without diffusing to the rest of the cell[8,9].

A long-debated issue concerns the heterogeneity of astrocytes. The debate has recently been advanced by the important identification of 'germinal astrocytes', which are GFAP-positive precursors that are concentrated in the neurogenic areas[10,11]. Moreover, mRNA microarray results have highlighted only a small number of genes that are ubiquitously expressed by astrocytes, whereas several other genes have been found only in the astrocytes of selected brain regions[12]. Functional studies of the hippocampus previously led to the distinction of astrocytic populations with different voltage-dependent current patterns (passive or complex[13]) and different responses to glutamate (currents that depend either on glutamate transporters or on AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors[14] (AMPARs).

Studies of mice that express enhanced green fluorescent protein (EGFP) under the control of the GFAP promoter[15] have confirmed that the cell population is heterogeneous[16–18]. About half the cells are rich in GFAP and show the typical properties of protoplasmic astrocytes: an irregular cell body with branched processes, low input resistance, a very negative membrane potential, voltage- and time-independent K^+ currents, prominent glutamate uptake and extensive dye coupling, which is indicative of gap-junctional coupling. By contrast, there is also a large population of fluorescent cells that is characterized by low GFAP expression, larger input resistance, lower electronegative membrane potential, voltage-dependent K^+ and Na^+ currents, AMPARs and low glutamate uptake. This population is not coupled through gap junctions[18]. Although these GFAP-poor cells are positive for S100 calcium-binding protein β (S100β), which is considered to be an astrocyte

marker, some also stain positively for the proteoglycan NG2 (REFS 16,17), a marker believed to be specific to a class of oligodendrocyte progenitor cells. The nature and role of NG2-positive cells remain elusive. They might be transitory cells or they might represent a previously unidentified class of glial cell, distinct from oligodendrocytes, microglia and probably also from classic astrocytes. Localized in both the white and the grey matter[19], NG2-positive cells receive direct synaptic inputs from neuronal fibres[20,21]. The significance of these inputs is unknown.

Multimodal control of the territory

The distribution of astrocytes in discrete territories properly positions them for local interactions. The idea that astrocytes connect blood vessels and neurons dates from Camillo Golgi's classic observations at the end of the nineteenth century[22]. However, the dynamic processes that complement these structural interactions — most notably the active dialogue between astrocytes and other brain elements — have only recently begun to emerge. According to this new perspective, astrocytes are highly polyvalent cells that are implicated in almost all processes that occur in the CNS, functioning as local integration units and bridges between synaptic and non-synaptic communication. Crucial roles in processes such as neurogenesis and synaptogenesis (for further information, see REFS 10,11,23–28) will be mentioned only briefly, as they are beyond the scope of this review. Here, we describe, with a few examples, diverse forms of interaction through which astrocytes control the activity of other cells in their territory.

Synaptic transmission can be modulated by dynamic changes in the astrocytic coverage of synapses. Indeed, the astrocyte–neuron partnership is not static but plastic, as has been directly documented by imaging studies of brain slices[6,7]. Astrocytic processes show spontaneous morphological changes in a matter of minutes, which reveals the dynamism of their interactions with synapses. This form of plasticity is probably important in the context of the contribution of astrocytes to brain signalling because, among other properties, the perisynaptic astrocytic processes express surface molecules that influence synaptic transmission. Such molecules include the excitatory amino acid transporters 1 and 2 (EAAT1/2)[29], which transport glutamate and control its extracellular concentration.

In the hypothalamic supraoptic nucleus, the morphological plasticity of astrocytes is related to a specific physiological state — lactation. Astrocytes of lactating animals retract their expansions from synapses that impinge on oxytocin-secreting neurons, which allows enlargement of their periplasmic space. A higher concentration of synaptically released glutamate can, therefore, escape glial reuptake, resulting in the stimulation of inhibitory metabotropic glutamate receptors (mGluRs) at both the presynaptic membrane[30] and the membrane of neighbouring GABA (γ-aminobutyric acid)-containing terminals[31]. This, in turn, leads to homo- and heterosynaptic depression of neurotransmitter release. At these and other synapses, the preferential diffusion of glutamate towards presynapses could depend on asymmetric astrocytic coverage, which is greater at the postsynaptic compartment[32].

The control of synaptic structure and function also depends on direct neuron–glia signalling. For example, in specialized cerebellar astrocytes known as BERGMANN GLIAL CELLS, an increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), which is induced by synaptic glutamate-dependent activation of AMPARs, is necessary to maintain the extensive coverage of synapses that impinge on Purkinje cells. Abolition of this glial signal induces alterations in synaptic currents and hyperinnervation of postsynaptic targets[33]. Similarly, in hippocampal CA1 pyramidal neurons, the binding of ephrin A3, which is found at the surface of astrocytic processes, to its receptor, EphA4, which is expressed on the membrane of dendritic spines, initiates signalling that regulates the number, size and shape of dendritic spines. EphA4-knockout mice have too many spines, which are both too long and largely disorganized[34].

Astrocytes also control non-neuronal brain cells. For example, they send rapid coordination signals to blood vessel cells that promote neurovascular coupling. They also attract cells to their territory through the release of chemokines, which are chemotactic agents that activate receptors on other cells. In this way, astrocytes coordinate the spatial positioning of oligodendrocytes during development[35], attract microglia and lymphocytes during inflammatory reactions and after injuries[36,37], and might drive reparative neural stem cells to lesion sites[38].

Astrocyte excitation

A turning-point in our understanding of astrocytes was triggered by the recognition of their active communication properties. Astrocytes are now viewed as 'excitable' cells in the sense that, when activated by internal or external signals, they deliver specific messages to neighbouring cells — an activity that has been dubbed 'gliotransmission'[39]. However, astrocytes cannot generate action potentials. Their excitation, which is chemically encoded, can be revealed not by electrophysiology, as in neurons, but by assays of $[Ca^{2+}]_i$ transients and oscillations. Two main forms of astrocyte excitation are well documented: one that is generated by chemical signals in neuronal circuits (neuron-dependent excitation) and one that occurs independently of neuronal input (spontaneous excitation).

Neuron-dependent excitation of astrocytes is widespread. First observed in the hippocampus and cerebellum (for reviews, see REFS 2,39), it has now been reported in many brain circuits following nerve fibre stimulation and the release of various transmitters and factors such as glutamate, GABA, acetylcholine, noradrenaline, dopamine, ATP, nitric oxide and brain-derived neurotrophic factor (BDNF)[40–47]. The transfer of information from neurons to glia occurs through the spill-over of synaptically released transmitters, and also by direct communication, such as that received by

BERGMANN GLIAL CELLS
(Also known as Golgi epithelial cells). These are the radial astrocytes of the cerebellum. Their highly branched processes make complex interactions with the synapses on Purkinje cell dendrites.

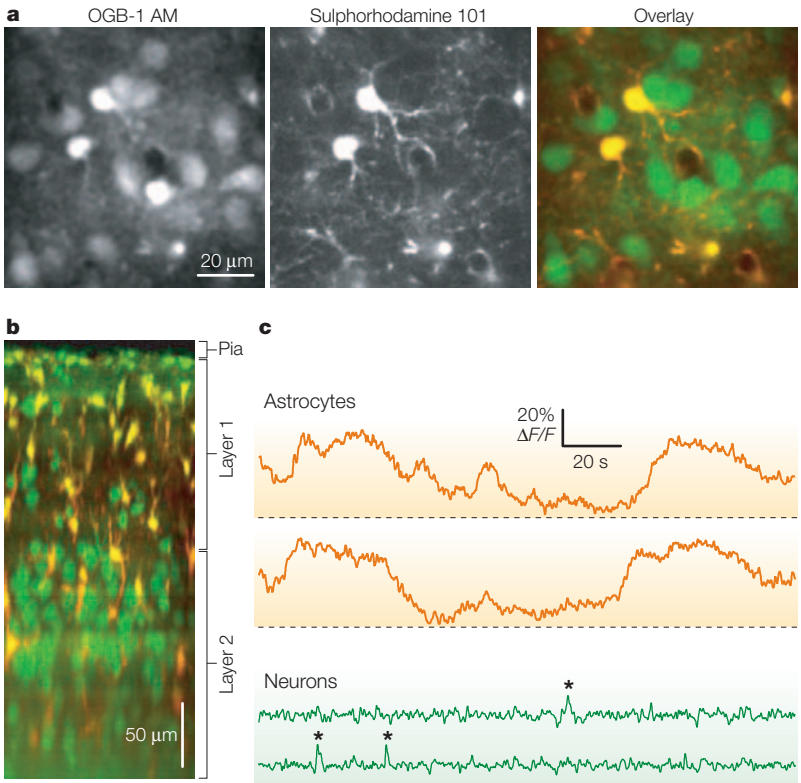


Figure 2 | **Simultaneous calcium imaging of astrocytic and neuronal networks *in vivo*.** **a** | Left panel shows two-photon fluorescence images of cells in layer 2/3 of rat neocortex, labelled using intracortical pressure ejection of the membrane-permeant green fluorescent calcium indicator dye Oregon Green 488 BAPTA-1 acetoxymethyl ester (OGB-1 AM). Central panel shows astrocytes counterstained with the astrocyte-specific red fluorescent dye sulphorhodamine 101 (REF. 59). Right panel shows an overlay of the green and red fluorescence images, which allows a clear separation of the astrocytic (yellow/orange) and neuronal (green) networks. Note that both astrocytes and neurons are stained by OGB-1 AM. **b** | Overview side-projection that shows astrocytes and neurons in layers 1 and 2 of rat neocortex. The image is a maximum-intensity side-projection from a stack of fluorescence images. **c** | Spontaneous intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) transients in astrocytes (orange traces) and neurons (green traces) measured as relative fluorescent change ($\Delta F/F$) over a time course of several minutes. Presumed neuronal $[Ca^{2+}]_i$ transients are indicated by asterisks above the traces. Note the different time course and amplitude of the astrocytic and neuronal transients. Figure modified, with permission, from REF. 59 © (2004) Macmillan Magazines Ltd.

cerebellar Bergmann glial cells from the ectopic presynaptic release sites of climbing fibres[48,49]. Although they coexist in the same nerve terminal, the ectopic and conventional release sites function independently and with different modalities, which ensures simultaneous, but distinct, communication to two postsynaptic cells, one of which is glial. Why does Bergmann glial cell excitation require the high temporal and spatial resolution of synapses? Tight coupling ensures high-fidelity activation of AMPARs, which are low affinity, fast-desensitizing receptors that are crucial for the control of climbing fibre–Purkinje cell synapses[33]. Tight coupling also largely overcomes glutamate uptake by Bergmann glial cell transporters, which predominates when stimulation occurs through glutamate spill-over from the cleft. These observations confirm the tripartite nature of many synapses[50,51], in which perisynaptic glia function as the third element of the signal-integration unit.

The properties of $[Ca^{2+}]_i$ oscillations generated in astrocytes, including their amplitude, frequency and propagation, are governed by the intrinsic properties of both neuronal inputs and astrocytes[40,42,47,52]. In Bergmann glial cells that are exposed to low-frequency parallel fibre stimulation, $[Ca^{2+}]_i$ increases are restricted to cell protrusions of a few cubic micrometres in volume that might represent independent functional microdomains[53]. However, when parallel fibres are stimulated at high frequency, several such microdomains are activated in a coordinated way. The ensuing $[Ca^{2+}]_i$ increases become generalized and propagate over longer distances, up to the cell body[40].

Astrocytes also discriminate neuronal inputs of different origins, and can integrate concomitant inputs[54]. Hippocampal astrocytes of the stratum oriens, which express both glutamatergic and cholinergic inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$)-generating receptors, respond with $[Ca^{2+}]_i$ elevations to stimulation of either Schaffer collaterals (which are glutamatergic) or nerve fibres in the stratum oriens/alveus (which are cholinergic and glutamatergic). Nevertheless, the response to the latter is mediated only by acetylcholine receptors; glutamate is taken up, but does not activate mGluRs. When released from stimulated Schaffer collaterals, however, glutamate induces mGluR-dependent $[Ca^{2+}]_i$ elevation. When Schaffer collaterals and stratum oriens/alveus fibres are stimulated simultaneously, the responses do not correspond to the sum of the $[Ca^{2+}]_i$ signals elicited by the separate stimulations, being either larger or smaller depending on the frequency of the stimulation (positive or negative cooperativity).

Spontaneous excitation is an unexpected property of astrocytes that has been observed both in acute brain slices[55–57] and *in vivo*[58,59]. It occurs in most astrocytes during development and decreases considerably during the first two postnatal weeks, when synaptic circuit formation occurs[55]. However, spontaneous astrocytic $[Ca^{2+}]_i$ oscillations do not disappear with adulthood[56,57]. They are generated by Ca^{2+} release from internal stores when $Ins(1,4,5)P_3$ receptors are activated, with additional influx of extracellular Ca^{2+}, possibly through voltage-gated channels[55–57]. These spontaneous intracellular Ca^{2+} signals are characterized by properties that are distinct from those in neurons, such as a large amplitude, long duration (tens of seconds) and regular, but infrequent, occurrence (from 0.5 to 5 min) (FIG. 2). Furthermore, they can either remain confined to distal processes that oscillate asynchronously or propagate to variable distances, intracellularly[56] or even intercellularly[55,59].

Importantly, spontaneous excitation of astrocytes can result in the excitation of neighbouring neurons[55], a finding that overturns the common idea that information is generated by neurons and travels through neuronal circuits before reaching the glia. However, $[Ca^{2+}]_i$ monitoring of large cell populations in brain slices and in the intact brain shows that both neurons and astrocytes are sources of excitation, and might operate in coordinated networks[57–59].

Table 1 | **The repertoire of gliotransmitters**

Gliotransmitter	Cellular storage site	Regulated release mechanism	Release stimulants and modulators	Site of action (receptors)	Cell targets and effects
Glutamate	SLMV, cytosol	Ca^{2+}-dependent exocytosis (activation of channels and/or transporters)	Glutamate, GABA, ATP, PG, TNFα, SDF1α, spontaneous astrocytic excitation	mGluR, AMPAR, kainate, NMDAR	Astrocytes, neurons (mostly stimulation)
ATP	?DCG, cytosol	Ca^{2+}-dependent exocytosis? (activation of channels and/or transporters)	ATP, glutamate, dopamine, LPA, thrombin	P2X, P2Y	Astrocytes, microglia, neurons, ?blood vessel cells (mostly stimulation)
Adenosine	Cytosol	Ectonucleotidase-mediated ATP dephosphorylation (activation of channels and/or transporters)	ATP, glutamate, dopamine, LPA, thrombin	A_1, A_2	Neurons (mostly inhibition)
D-serine	?SLMV	Ca^{2+}-dependent exocytosis	Glutamate	NMDAR (glycine site)	Neurons (stimulation)
Eicosanoids (PG, HETE)	Not known to be stored	Ca^{2+}-dependent synthesis followed by rapid release	Glutamate, TNFα, SDF1α, noradrenaline	Eicosanoid receptors	Astrocytes, blood vessel cells (stimulation, vasodilation/ contraction)
Cytokines (TNFα)	Cell surface	Ca^{2+}-dependent, TACE-mediated surface proteolysis	SDF1α	TNFα receptors	Astrocytes, neurons (stimulation)
Proteins and peptides (AchBP[152], ANP, ?others)	DCG	Ca^{2+}-dependent exocytosis	Ach (for AchBP)	Ach binding, ANP and other peptide receptors	Neurons (AchBP; inhibition)

Gliotransmission was first revealed in 1994 when increases in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in cultured astrocytes were shown to induce glutamate release followed by neuronal activation[149]. However, a precise definition of gliotransmitters is missing. Here, we propose the following criteria for molecules released by astrocytes: first, synthesis by and/or storage in the astrocytes; second, regulated release triggered by physiological stimuli; third, activation of rapid (milliseconds to seconds) responses in neighbouring cells; and fourth, a role in physiological processes. Only the agents that meet these criteria have been included. The release of glutamate, ATP and adenosine through channels and/or transporters is given in brackets because its occurrence in physiological conditions is still questioned. Several other agents, including taurine[150] and homocysteic acid[151], have been proposed to function as gliotransmitters; however, the evidence for these is still inconclusive. Therefore, they have not been included in this table. A_1, A_2, adenosine receptors; Ach, acetylcholine; AchBP, acetylcholine-binding protein; ANP, atrial natriuretic peptide; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid glutamate receptor; DCG, dense-core granule; GABA, γ-aminobutyric acid; HETE, 20-hydroxyeicosatetraenoic acid; LPA, lysophosphatidic acid; mGluR, metabotropic glutamate receptor; NMDAR, *N*-methyl-D-aspartate glutamate receptor; P2X, P2Y, purinergic 2X and 2Y receptors; PG, prostaglandin; SDF1α, stromal-derived factor-1α (a chemokine); SLMV, synaptic-like microvesicle; TACE, TNFα-converting enzyme; TNFα, tumor necrosis factor-α.

Many questions that concern excitation remain open. First, the range of propagation of intracellular Ca^{2+} signals under physiological conditions remains undefined. Electrical, mechanical and chemical stimulation of astrocytes generates intercellular Ca^{2+} waves of variable extension, from microcircuits involving small groups of cells[60] to multicellular pathways that are hundreds of micrometres long[47,61–63]. Do these responses represent physiological, pathological or just artificial processes? Furthermore, how do Ca^{2+} signals travel from one astrocyte to another?

Two main routes, initially observed in cell cultures, have recently been confirmed in acute slice preparations: one intracellular, probably involving the cell-to-cell diffusion of $Ins(1,4,5)P_3$ through gap-junction channels[61,63]; the other extracellular, involving the release of factors such as ATP[61–63]. However, the specificities of the two routes are unknown. Moreover, the biochemical events associated with astrocyte excitation and signal propagation are probably more complex and relevant than is currently understood. In cultured astrocytes, waves of extracellular glutamate[64] have been reported to occur in parallel with waves of Ca^{2+} that are also accompanied by cytosolic Na^+ waves. The latter have been termed 'metabolic waves', because they reveal concerted uptake of glutamate and glucose, which might underlie the spatial coordination of neuro-metabolic coupling[65]. Finally, little is known about the influence of environmental factors on astrocyte excitation, except that in cultured astrocytes the addition to the medium of growth factors, cytokines or combinations of these was found to induce important changes in the nature and propagation of intracellular Ca^{2+} signals[66,67].

Regulated gliotransmitter exocytosis

An important response of astrocytes to their excitation, both by neuronal input and by self-generated stimuli, is the release of gliotransmitters — chemicals that act on adjacent neurons, glial cells and vessels. Over the years, the number of proposed gliotransmitters has increased, and their properties have, in part, been unravelled (TABLE 1). Here and in the following section, we focus on the mechanisms of their release.

Exocytosis — the rapid form of transmitter release that is typical of neurons and neurosecretory cells — consists of the fusion of specific, membrane-bound vesicles with the plasma membrane, followed by the quantal discharge of their content. Whether a process of this type also occurs in astrocytes and accounts, at least in part, for gliotransmission has long been debated. A large body of indirect supporting evidence, including the identification of crucial components of the exocytotic machinery and of Ca^{2+}-dependent release processes that are sensitive to blockers and stimulants of neuronal exocytosis (for reviews, see REFS 68,69) was counterbalanced by the lack of evidence for a specific vesicular population that was competent for regulated exocytosis[70]. Recently, however, a clear synaptic-like microvesicle (SLMV) compartment, which is equipped for the uptake, storage and release of glutamate, has been identified in adult hippocampal astrocytes, and Ca^{2+}-dependent exocytotic fusion has been documented in astrocyte cultures by total internal reflection fluorescence imaging[71] (see BOX 1 and Supplementary information S1 (movie)) and membrane capacitance measurements[72,73].

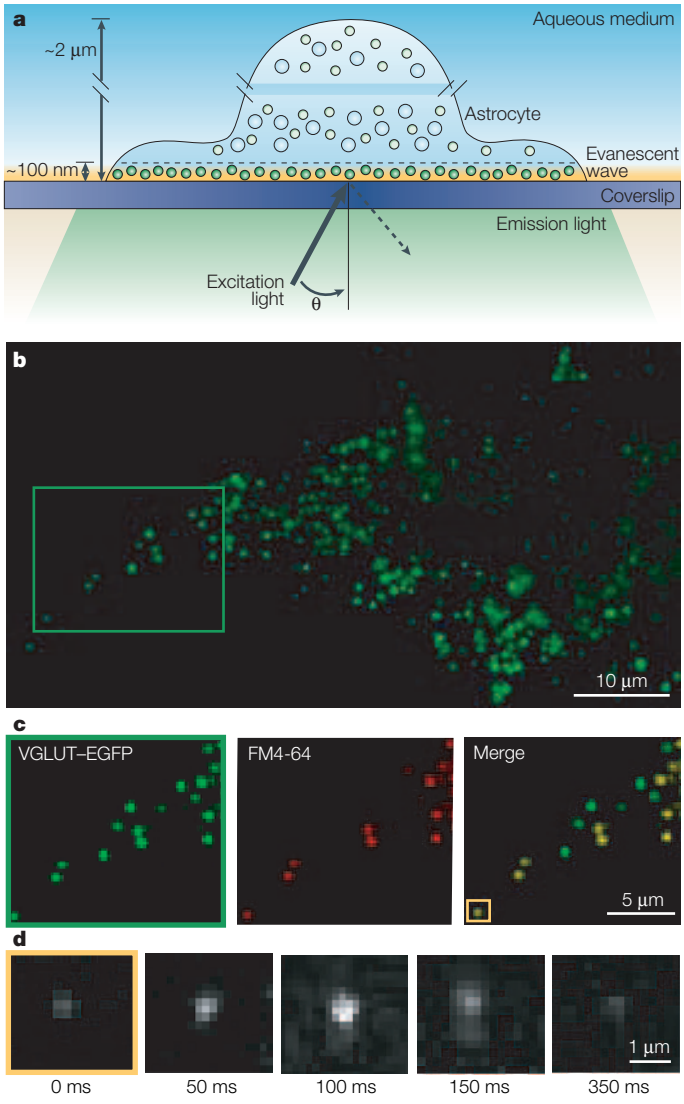
Morphologically, astrocytic SLMVs resemble glutamatergic synaptic vesicles, although they have a much less dense and orderly arrangement. In perisynaptic cell expansions, small, loosely arranged SLMV groups are often distributed just beneath the plasma membrane, opposite neuronal terminals or dendrites, and sometimes within synaptic distance of neuronal NMDA (*N*-methyl-D-aspartate) receptors (NMDARs)[71] (FIG. 3). Astrocytic SLMVs express proteins that govern exocytosis — that is, the R-type SNAP (soluble N-ethylmaleimide-sensitive fusion protein attachment protein) receptor (R-SNARE) vesicle-associated membrane protein 3 (VAMP3, also known as cellubrevin), together with vesicular glutamate transporters (VGLUTs)[71]. The three known VGLUT isoforms[74] (VGLUT1–3) are all expressed by astrocytes, although not by all astrocytes[71,72,75]. This indicates that there are either VGLUT-positive and VGLUT-negative astrocytes or that various VGLUT isoforms are distributed over distinct subpopulations of astrocytes, as has been observed in neurons[74].

Functionally, astrocytic exocytosis has peculiar properties. Although an accurate comparison is not yet possible, the events seem to be significantly slower and the Ca^{2+} affinity of the release machinery higher than at neuronal synapses[71,73]. Gliotransmitter release is triggered not by the action potential-induced opening of specific presynaptic Ca^{2+} channels, but by the activation of G-protein-coupled receptors (GPCRs), with ensuing Ins(1,4,5)P_3-induced Ca^{2+} release from the stores of the endoplasmic reticulum. Among GPCR-evoked processes in various cell types[76], mGluR-dependent exocytosis of SLMVs in astrocytes is perhaps the fastest[71], which indicates that endoplasmic reticulum cisternae are close to the vesicular release sites[71,77].

Differences between gliotransmitter and neurotransmitter release might also arise from their different exocytotic machinery. Single-cell RT-PCR (polymerase chain reaction after reverse transcription) studies, performed *in situ* and in freshly isolated astrocytes, and high-resolution immunocytochemistry have documented the expression of four exocytotic proteins: SNAP23, a synaptic Q-SNARE; complexin 2; Munc18a; and synaptotagmin IV. By contrast, SNAP25 and the synaptic vesicle proteins synaptotagmin I, synaptophysin and synaptic vesicle glycoprotein 2 (SV2) were not found[72,78,79]. Functionally, VAMP3 can be a substitute for VAMP2 (REFS 80–82), the expression of which in tissue astrocytes remains controversial[71,72,80]. Furthermore, SNAP23, instead of SNAP25, could enhance the Ca^{2+} sensitivity of exocytosis[82,83]. The role of synaptotagmin IV, present instead of synaptotagmin I, cannot be easily deciphered. Its contribution to the Ca^{2+}-sensing mechanism has been questioned[84], but it has been reported to favour a kiss-and-run mechanism — that is, the incomplete exocytotic fusion associated with fast vesicle recycling[85].

Together with SLMVs, larger (100–700 nM), heterogeneous organelles[86] are present in cultured astrocytes, some of which contain secretogranin II (REF. 87), a typical marker of neuronal dense-core secretory granules, and ATP[88]. Therefore, nucleotides and peptides, such as ANP (atrial natriuretic peptide), could be released from organelles that are distinct and regulated differently from glutamatergic SLMVs[88,89]. By contrast, D-serine, a potent co-agonist of glutamate at the NMDAR glycine-binding sites, was recently reported to co-localize with markers of SLMVs, and could, therefore, be co-released from the vesicle population that is responsible for glutamate release[90]. Functionally, this combination could be highly effective in activating NMDARs. These observations, although potentially important in view of the coordinate or antagonistic functional roles of the different gliotransmitters, should be interpreted with caution because *in vitro* cultures only partially reproduce the specific gliotransmission properties of astrocytes in their native tissue environment. For example, VGLUT heterogeneity disappears *in vitro*, and all cultured astrocytes express multiple isoforms of the transporter together with various secretion-related proteins that are absent from tissue astrocytes[71–73,75,91]. In addition, the levels of some of these proteins change with time in culture[79]. Studies of preparations *in situ* will therefore be necessary to confirm and clarify the heterogeneity and specific properties of the regulated exocytotic pathways in astrocytes.

Box 1 | **Imaging glutamate exocytosis from astrocytes**



Exocytotic fusion is studied using two main approaches: patch-clamp recordings of membrane capacitance, and dynamic imaging, notably total internal reflection fluorescence microscopy (TIRFM)[148]. The latter was used to show regulated glutamate exocytosis from astrocytes[71]. With TIRFM, vesicle populations can be directly visualized, identified using specific markers and studied dynamically with single-vesicle resolution.

To highlight glutamate-containing vesicles, cultured astrocytes were engineered to express the vesicular glutamate transporters (VGLUTs), which were tagged with enhanced green fluorescent protein (EGFP). Panel a illustrates the basic principle of TIRFM: the excitation light is discriminated according to the angle of incidence (θ), so that only an evanescent wave (yellow) penetrates into a cell by ≤100 nm, where it selectively illuminates the fluorophores in this thin superficial layer.

Panel b shows the fingerprint image of the TIRF-illuminated cell layer (green emission light), revealing a homogeneous population of VGLUT-containing vesicles that are docked to or immediately adjacent to the plasma membrane. Such vesicles comprise only a minority of the total vesicle population in an astrocyte.

Panel c shows exocytosis of glutamatergic vesicles, which was studied by combining VGLUT–EGFP with a red fluorescent dye indicator — either acridine orange (AO, see Supplementary information S1 (movie)) or FM4-64 — and by monitoring the red and green fluorescence emissions. Both AO and FM4-64 accumulate in the vesicles, and their release, which is accompanied by a change in fluorescence, signals the exocytotic event. FM4-64 partitions into, but does not penetrate, membranes. Therefore, its fluorescence reveals membranes that have contacted the extracellular fluid and identifies the recycling pool of VGLUT–EGFP-positive vesicles (yellow dots in 'merge').

The temporal sequence in panel d shows exocytosis of an individual VGLUT-positive vesicle, seen as FM4-64 destaining in response to an increase in the intracellular Ca^{2+}concentration ($[Ca^{2+}]_i$) induced by ionomycin. Fluorescence initially spreads over the plasma membrane (third image in panel d), and subsequently declines. Images courtesy of P. Bezzi, Department of Cell Biology and Cellular Imaging Facility, University of Lausanne, Switzerland.

Exocytotic gliotransmission is probably not a simple consequence of increases in $[Ca^{2+}]_i$. In fact, several distinct stimuli that all induce large $[Ca^{2+}]_i$ responses evoke different transmitter release responses[92,93]. Moreover, in some cases, GPCR-induced glutamate release can be suppressed by blocking the synthesis of prostaglandins, tumour necrosis factor-α (TNFα) or both[94–96]. However, the role of these mediators is unclear. Prostaglandins could act as autocrine and/or paracrine amplifiers of GPCR-induced $[Ca^{2+}]_i$ increases through stimulation of their own receptors[94,97], although some reports have called for alternative mechanisms, as the suppression of prostaglandin synthesis does not, apparently, modify $[Ca^{2+}]_i$ responses[96,98].

Interestingly, gliotransmitter release can be modified by concomitant activation of distinct receptors, with ensuing positive or negative cooperativity.

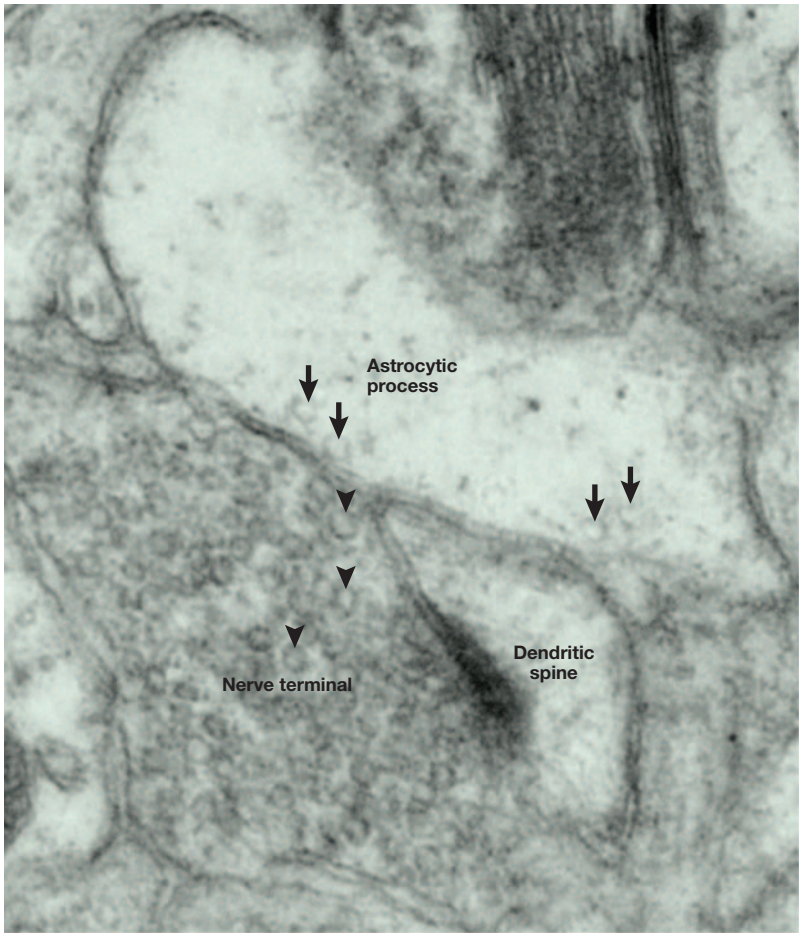


Figure 3 | **Synaptic-like microvesicles in an astrocyte process facing an excitatory synapse in the hippocampus.** Electron micrograph showing synaptic-like microvesicles (SLMVs) in an astrocytic process in the outer two-thirds of the hippocampal dentate molecular layer. Arrows indicate astrocytic SLMVs. These vesicles resemble synaptic vesicles (arrowheads) in both shape and size, and are observed in close proximity to the asymmetric synaptic specialization, at extrasynaptic sites that face either the nerve terminal or a dendritic spine. To obtain better morphological preservation than that previously obtained using tissue prepared with Lowicryl for immunogold detection of vesicular glutamate transporters (VGLUTs) and SNARE proteins[71], the tissue was perfusion-fixed with a mixture of 2.5% glutaraldehyde and 1% formaldehyde, and postfixed with 1% osmium tetroxide before being embedded in Durcupan (Fluka AG, Switzerland). Micrograph courtesy of V. Gundersen, Anatomical Institute, University of Oslo, Norway (unpublished observations).

For example, activation of AMPARs, which does not stimulate glutamate release *per se*, potentiates mGluR-evoked release and strengthens mGluR-dependent prostaglandin E_2 (PGE_2) production[94], possibly through transactivation of epidermal growth factor receptors (ErbB receptors)[99]. By contrast, the interaction between mGluRs and α_1-noradrenergic receptors is an example of negative cooperativity. The $[Ca^{2+}]_i$ elevations induced by the latter fail to stimulate glutamate release, but instead suppress a coincident mGluR-evoked release[92]. To conclude, the stimulus-secretion coupling mechanisms in astrocytes are only partially understood. In addition to Ca^{2+} signals with specific spatiotemporal encoding, they seem to comprise transduction events of a different nature that are triggered by receptor activation.

VOLUME-REGULATED ANION CHANNELS (VRACs). Channels activated not by voltage changes or ligand binding but by the swelling of the cell. They are permeant to monovalent anions and organic osmolytes, such as amino acids and polyols.

Non-exocytotic gliotransmitter release

Molecular transport across the plasma membrane through specialized proteins such as channels and transporters represents a second modality by which hydrophilic agents can be released from cells.

Three types of large plasma membrane channel have been claimed to participate in the non-exocytotic release of gliotransmitters, in particular of ATP and glutamate. The existence of specific VOLUME-REGULATED ANION CHANNELS (VRACs) was deduced from the observation that anionic amino acids, such as glutamate, aspartate and taurine, are released in a non-vesicular, Ca^{2+}-independent fashion when astrocytes swell, notably in hypotonic media[100] (but see REF. 101 for a possible example of Ca^{2+}-induced modulation). The molecular identity of VRACs is still unknown, and the involvement of other channels cannot be excluded.

GAP-JUNCTION HEMICHANNELS, which are formed by hexamers of connexin 43 (CX43), assemble in astrocytes and might function as autonomous permeation pathways[102]. The opening probability of the hemichannels, which is very low in astrocytes bathed in physiological media, increases significantly by reducing the concentrations of divalent cations, notably Ca^{2+}, and is accompanied by the release of both ATP[103,104] and glutamate[105]. The involvement of hemichannels is consistent with the suppression of the release of these gliotransmitters by gap-junction channel blockers and, in the case of ATP, the absence of release in a glial cell line that lacks CX43, with restoration after transfection of the connexin.

The PURINERGIC $P2X_7$ RECEPTOR, which has been proposed to be a source of glutamate and D-aspartate release[106], shares some properties with hemichannels, including increased opening probability at low extracellular Ca^{2+} concentrations. However, its pharmacology is different: it is activated by ATP at high concentrations (EC_{50} = 300 μM), by 3′-O-(4-benzoyl-benzoyl) ATP (BzATP) at lower concentrations, and can be blocked by oxidized ATP but not by suramin[107]. It is not clear whether the high ATP concentrations needed for $P2X_7$ gating can be reached extracellularly in the healthy brain[44,93,108]. Alternatively, prolonged activation of $P2X_7$ channels in pathological conditions could lead to the release of cytosolic proteins and cell death[107,109].

Among plasma membrane transporters, the ATP-BINDING CASSETTES (otherwise known as multidrug resistance transporters) have been proposed to account for the swelling-induced release of ATP[110]; however, the evidence is still preliminary. Reversal of HIGH-AFFINITY GLUTAMATE TRANSPORTERS is unlikely to occur during normal brain function[29,111]. Nevertheless, glutamate could be released by the CYSTINE/GLUTAMATE EXCHANGER[112], a candidate for the cytosolic accumulation of cystine that is necessary for the synthesis of glutathione, one of the main endogenous antioxidants. Whether the extracellular concentrations of endogenous cystine are high enough to trigger the exchanger activity and whether its glutamate release occurs from astrocytes is unclear[113].

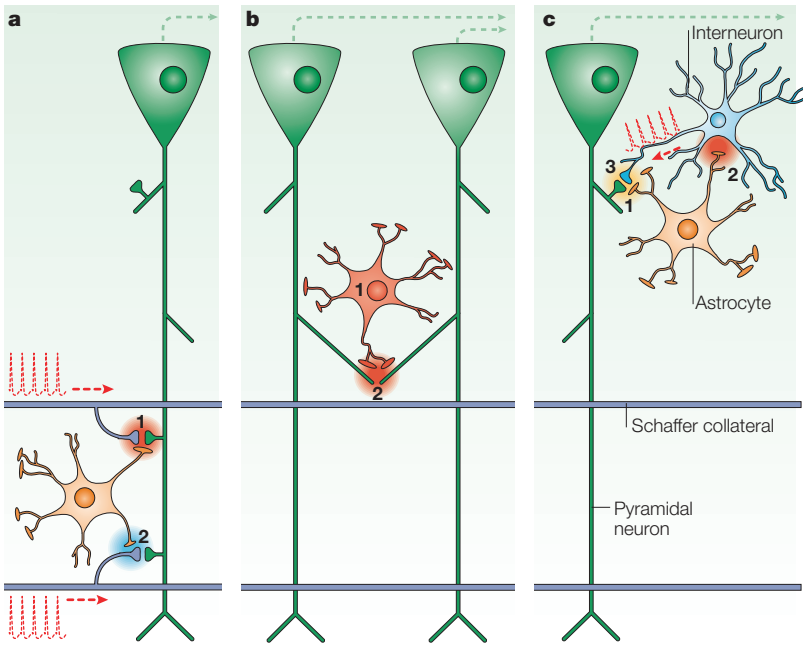


Figure 4 | **Astrocytes exert multimodal control on synaptic transmission and neuronal excitability in the CA1 region of the hippocampus.** The drawing depicts three distinct actions of astrocytes on the function of CA1 pyramidal neurons (PNs). **a** | Heterosynaptic depression of synapses between Schaffer collaterals (SCs) and PNs — an example of feedforward synaptic modulation. Glutamate released (1; red spot) at a high-frequency-activated SC–PN synapse stimulates an interposed astrocyte, which responds by releasing ATP. This is rapidly converted to adenosine (2; blue spot), which induces suppression of a different SC–PN connection through the activation of a presynaptic A_1 adenosine receptor. **b** | Excitation and synchronization of adjacent PNs — an example of bridging non-directly connected neuronal circuits. Spontaneous oscillations in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in an astrocyte (1) trigger glutamate release (2; red spot), which is sensed simultaneously by two contiguous PNs. This leads to the generation of synchronous NMDAR-dependent excitatory currents. **c** | Potentiation of inhibitory synapses between GABA (γ-aminobutyric acid)-containing interneurons (GIs) and PNs — an example of feedback synaptic modulation. GABA that is released (1; yellow spot) by repetitive firing at GI–PN synapses activates $GABA_B$ (GABA type B) receptors on a neighbouring astrocyte, which responds by releasing glutamate (2; red spot) onto the GI. This causes feedback potentiation of the GI's inhibitory drive (3) on the PN.

In conclusion, current evidence indicates that some gliotransmission might occur through channels and transporters; however, many aspects of these processes need to be clarified. Most importantly, we need to determine whether these processes occur under physiological conditions and how relevant they are, especially because no mechanisms that account for their specificity and regulation have been identified. The results of numerous studies in the field are inconclusive because of poor pharmacology (for a review, see REF. 68) and/or because the available information about the participating transport proteins is insufficient.

The physiology of astrocytic communication

Astrocytes respond to excitatory external inputs and to spontaneous excitation by generating feedback or feedforward messages (FIG. 4). They can function as bridging units through feedforward messages, linking cells and structures such as nearby neurons, blood vessel cells and other glia that are not directly connected to each other. What are the functional consequences of these interactions?

Modulation of synaptic transmission. Modulation of neuronal excitability and synaptic transmission by astrocytes was first shown to be mediated by glutamate release (for a review, see REF. 2). Recently, modulatory effects mediated by ATP and its derivative, adenosine, have also emerged[44,47,108]. Interestingly, purines and glutamate were found to have opposite effects in the same synaptic territory: inhibitory for purines[44] and stimulatory for glutamate[114]. Astrocytes could, therefore, exert non-stereotyped, bimodal synaptic control through the release of these gliotransmitters. Moreover, as both glutamate and purines have many receptor targets on both excitatory and inhibitory neurons, and as astrocytes also release additional gliotransmitters (TABLE 1), the range of astrocyte–synapse interactions might be even more complex.

So far, the experimental models that have been most intensely investigated are the retinal and hippocampal circuits. Activation of retinal glia — astrocytes and radial Müller cells — modulates the light-evoked spiking activity of ganglion cells that project to the brain, thereby affecting the processing of visual information[115]. Two opposite effects have been identified[108,116]. The first is inhibitory and is mediated by ATP when it is released from stimulated Müller cells. ATP is then rapidly transformed into adenosine by the dephosphorylating action of the ectonucleotidases anchored to the plasma membrane, probably near the ATP release sites[93]. Adenosine inhibits ganglion cells through A_1 adenosine receptors by the activation of a hyperpolarizing K^+ conductance[108]. The second effect is stimulatory and is mediated by D-serine acting at the glycine-binding site of the NMDAR. In the retina, D-serine is present exclusively in Müller cells and astrocytes. Its release and physiological action during excitatory synaptic activity were revealed through inhibition induced by the D-serine-degrading enzyme, D-amino acid oxidase, in the NMDA component of light-evoked ganglion cell excitatory postsynaptic currents[116].

Excitatory synaptic transmission is also subject to antagonistic modulatory influences from astrocyte-released gliotransmitters at the Schaffer collateral–pyramidal cell synapses of the CA1 region of the hippocampus. Gliotransmitters act on excitatory group I mGluRs and inhibitory A_1 adenosine receptors of neuronal afferents, which produces presynaptic facilitation (glutamate)[114] or inhibition (adenosine)[44], respectively. Moreover, the high-frequency activity of a Schaffer collateral fibre can trigger the heterosynaptic suppression of another, adjacent fibre. This process is not due to neurotransmitter spill-over from a first to a second synapse, as has generally been thought, but to the feedforward modulation of an intermediate astrocyte that senses the level of activity in the first fibre and consequently tunes the activity of a second[44] (FIG. 4a).

Astrocytes also control inhibitory synaptic transmission. The best-characterized example of this is the potentiation of hippocampal synapses between the GABA-containing interneurons of the stratum radiatum and CA1 pyramidal neurons, which is established in response to repetitive firing of interneurons[117].

GAP-JUNCTION HEMICHANNELS
(Also known as connexons). Large, non-selective ion channels composed of connexin subunits. They can reside in the plasma membrane autonomously, or be coupled with a hemichannel of an adjacent cell to form a gap junction.

PURINERGIC $P2X_7$ RECEPTOR
A plasma membrane channel that is activated by the binding of ATP and is permeant to mono- and divalent cations. In many cells, on sustained stimulation, the aqueous pore dilates to admit larger molecules irrespective of their charge.

This effect is not direct but depends on the activation of neighbouring astrocytes by GABA. The feedback release of glutamate by astrocytes decreases GABA-mediated synaptic failures, possibly through the stimulation of GluR5-containing kainate receptors on the interneurons[118] (FIG. 4c). Not all astrocytic modulations of hippocampal interneurons are mediated by glutamate. Other forms of modulation might occur through the release of ATP, which results in purinergic 2Y (P2Y) receptor activation[47].

Neuronal synchronization. Astrocytes are also involved in the stimulation of postsynaptic neuronal excitability[46,54,55,119]. In hippocampal slices bathed in Mg^{2+}-free medium, CA1 pyramidal neurons show transient, slow inward currents (SICs) that are kinetically distinct from synaptic currents and are often of large amplitude. Such currents, which are sustained purely by NMDAR activation and are unaffected by the blockade of synaptic transmitter release, depend on the spontaneous $[Ca^{2+}]_i$ oscillations of astrocytes, and their frequency (<1 min^{-1}) increases following the stimulation of astrocytes[46,54,119]. Most importantly, single SICs can occur with delays of <100 msec in two (and probably more) contiguous pyramidal neurons, provided that they are separated by a distance of no more than 100 μm[46,119]. A probable explanation for this is that $[Ca^{2+}]_i$ elevation triggers an episode of glutamate release in one astrocyte that is sensed simultaneously by the pyramidal cells in that astrocytic territory. By inducing synchronized SICs, a single astrocyte therefore functions as a bridging unit between circuits that are not directly connected to each other (FIG. 4b). Synchronization of neuronal activity has been proposed to contribute to information processing in the brain[120]. Can astrocyte-induced SICs be a relevant underlying mechanism? On the one hand, SICs have been observed not only in Mg^{2+}-free, but also in normal Mg^{2+} conditions[46], in the hippocampus as well as in other brain areas, with amplitudes sufficient to drive neurons to fire action potentials[55]. On the other hand, under physiological conditions, their frequency is low (about once every 2 min, when astrocytes are stimulated), which makes it difficult to predict what contribution they could make to rapid neuronal synchronicity.

Recordings of SICs under physiological conditions indicate that the Mg^{2+} blockade of NMDARs can be overcome without apparent AMPAR activation. This could occur because extrasynaptic NMDARs that are exposed to astrocyte-released glutamate[71] have a peculiar subunit composition. Alternatively, it might occur through astrocyte-released glutamate activation of other excitatory amino acid receptors that are responsible for membrane depolarization, or because astrocytes co-release with glutamate-depolarizing agents and/or facilitatory factors that are specific to NMDARs, such as D-serine. Indeed, D-serine released from astrocytes at CA1 pyramidal cell synapses was reported to modulate NMDAR-dependent long-term potentiation[121]. The strength of the same synapses is under the tonic control of astrocyte-released TNFα[122]. These observations implicate gliotransmitters in synaptic plasticity.

Regulation of cerebral blood flow. More than 100 years ago, it was proposed that cerebral blood flow adapts to neuronal activity (cerebrovascular coupling) and that vascular tone depends on the release of vasoactive agents into the perivascular space. However, a causal relationship between $[Ca^{2+}]_i$-dependent astrocyte gliotransmission and variations in arteriolar tone was documented only recently, by three studies of brain slices[9,45,123]. In the first study, neuronal activity-dependent astrocytic $[Ca^{2+}]_i$ oscillations were shown to induce vasodilation through the release of cyclooxygenase eicosanoids, probably PGE_2 (REFS 45,98). By contrast, in the second study, the increase in astrocytic $[Ca^{2+}]_i$, which was produced by photolysis of caged Ca^{2+} and propagated at the vascular interface from end-foot to end-foot, induced rapid constriction of the underlying vascular region, mediated by a different eicosanoid, 20-hydroxyeicosatetraenoic acid (20-HETE)[9] (see Supplementary information S2 (movie)). Finally, in the third study, astrocytic $[Ca^{2+}]_i$ elevations suppressed the $[Ca^{2+}]_i$ oscillations in vascular myocytes and the ensuing rhythmic fluctuations in arteriolar diameter[123]. Taken together, these results indicate that astrocyte activation can induce distinct responses depending on the functional state of vessels: for example, vasodilation was observed mostly using preconstricted vessels, and vasoconstriction using normal vessels. In all cases, slice preparations could only partially reproduce the conditions of physiological circulation, because the vessels lacked luminal blood flow and endogenous tone.

Active role of astrocytes in brain pathology

The role of astrocytes in neurodamaging processes — and notably their reaction to neuronal injury (reactive gliosis, developed together with microglia) — has long been known. Until recently, however, no specific role had been identified for astrocytes in the pathogenesis of discrete CNS diseases. Now, alterations in the partnership between astrocytes and neurons are beginning to emerge as important mechanisms that underlie brain lesions. We first discuss several defects in astrocyte excitation and signalling that have been observed in the course of pathology. We then focus on four distinct diseases in which astrocytes seem to be crucial for pathogenesis.

Defects in excitation and signalling. Almost all the steps of astrocyte excitation and gliotransmission are affected in various pathological conditions. Spontaneous $[Ca^{2+}]_i$ oscillations, by which astrocytes stimulate their glutamate release and synchronize neighbouring neurons[46,55,57–59,119], are abolished in the peritraumatic area of mechanical insults[57] and are stimulated by epileptiform neuronal activity[57,58]. Moreover, network interactions between astrocytes and neurons take place in the spreading depression that underlies the aura of migraine, a slowly-propagating, long-range

ATP-BINDING CASSETTES (ABC proteins). A superfamily of integral membrane proteins that bind and hydrolyse ATP. Most function to translocate specific substrates across cell membranes.

HIGH-AFFINITY GLUTAMATE TRANSPORTERS
A family of proteins in the plasma membrane of astrocytes and neurons with the specific function of removing glutamate from the extracellular fluid. For each transport cycle, together with one glutamate molecule, they co-transport three Na^+ ions and one H^+ ion, and countertransport one K^+ ion.

CYSTINE/GLUTAMATE EXCHANGER
A Na^+-independent amino acid antiporter that exchanges extracellular cystine for intracellular glutamate. These exchangers are ubiquitous in brain cells, and each comprises two separate proteins: a light chain that confers specificity, and a heavy chain.

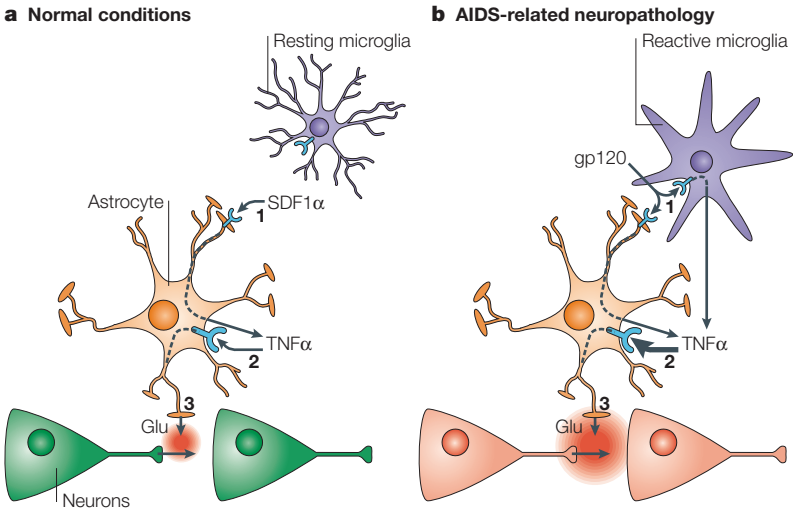


Figure 5 | **Microglia-dependent transformation of CXCR4-evoked gliotransmission into a neurotoxic pathway in AIDS-related neuropathology.** Proposed sequence of cellular interactions and signalling events in normal physiological conditions (**a**) and in AIDS-related neuropathology (**b**). **a** | In astrocytes, binding of the chemokine stromal-derived factor-1α (SDF1α) to its specific receptor, chemokine (C-X-C motif) receptor 4 (CXCR4; 1), triggers intracellular events that result in the extracellular release of soluble tumour necrosis factor-α (TNFα). The interaction of TNFα with its cell-surface receptors (2) initiates a second sequence of intracellular signalling events that leads to Ca^{2+}-dependent glutamate (Glu) release (3; red spot). This, in turn, induces modulatory effects at neuronal synapses[133]. Microglia are in the resting state and do not participate in the astrocyte–neuron signalling. **b** | In AIDS-related neuropathology, microglia that are infected by HIV become reactive. They can come in close apposition to astrocytes and shed HIV particles, including the envelope glycoprotein gp120. gp120 of T-tropic HIV stimulates CXCR4-dependent signalling in both astrocytes and microglia simultaneously (1). As a result, the two cell types contribute synergistically to the release of high levels of TNFα, with enhanced activation of TNFα receptors in astrocytes (2), which ultimately results in massive glutamate release (3; red spot). This causes neuronal damage and apoptosis.

wave of neuronal depolarization that is accompanied by an astrocytic Ca^{2+} wave[63]. Hypoxic insults to cultured astrocytes change the state of Ca^{2+} stores, and, in particular, the buffering capacity of mitochondria, so that responses to external inputs are profoundly altered[124].

Gliotransmission in the hippocampus is prominently stimulated by glutamatergic activation of astrocyte mGluRs[45,46,94,119] and/or AMPARs[44,94,121]. In patients with epilepsy who have AMMON'S HORN SCLEROSIS, a splicing alteration in the GluR1 subunit might increase AMPAR-dependent responses and favour astrocyte hyperexcitability[125]. It is even more striking that after an ischaemic insult NMDARs appear in hippocampal astrocytes, which normally lack these receptors, and persist for a few weeks, generating specific Ca^{2+} signals. These changes might compensate for the inactive NMDARs of affected neurons and help to prevent, at least in part, the degeneration of afferent fibres[126]. Alterations in Ca^{2+}-dependent glutamate release occur in response to acute glial inflammation. The clustering of astrocytes together with pathology-activated microglia gives rise to anomalous interactions between the two cell types, which leads to massive TNFα release. This, in turn, amplifies astrocytic glutamate release and triggers its conversion to a neurotoxic process[95].

AMMON'S HORN SCLEROSIS
A brain lesion that is characterized by neuronal loss and reactive gliosis — which forms a 'scar' — localized in Ammon's horn of the hippocampus.

Conditions such as ischaemia and traumatic injury favour the release of gliotransmitters, particularly through Ca^{2+}-independent pathways. For example, CX43 hemichannels become highly permeable in pseudo-ischaemic conditions[127]. However, the role of released gliotransmitters in the development of ischaemic or traumatic brain damage is unclear. In hippocampal slices that have been exposed to simulated ischaemia for a few minutes, astrocytic release processes, such as Ca^{2+}-dependent exocytosis and VRAC-mediated glutamate release, have no apparent role in the onset of the neuronal depolarization that blocks information processing. This latter effect is mainly due to glutamate release through the reversed operation of its transporters, which occurs in neurons but not in astrocytes[111]. However, the ensuing overstimulation of NMDARs, which leads to neuronal toxicity, is exacerbated by the concomitant release of D-serine from astrocytes[128]. In a model of spinal cord injury, excitotoxic neuronal damage was found to be mediated mainly by excessive ATP release with overstimulation of $P2X_7$ receptors[109], although the cellular origin of the purine was not established.

Brain tumours. Gliomas, which are derived from the malignant transformation of astrocytes and other glial cells, are a family of aggressive brain tumours that gain space for growth through the destruction of surrounding tissues and the invasion of new territories[129]. One mechanism of expansion is the release of excess glutamate, possibly through a Ca^{2+}-independent pathway, with ensuing NMDA-dependent neuronal excitotoxicity[129,130]. In the most aggressive tumours, this excess release of glutamate is combined with a lack of glutamate uptake, which leads to even higher extracellular accumulation and neurotoxicity. In addition, gliomas express AMPARs that lack the GluR2 subunit and are therefore permeable to Ca^{2+}. This is an essential property for proliferation and migration, as shown by forced expression of the subunit, which inhibits tumour cell locomotion and induces apoptosis[131]. Through their modified glutamate signalling, glioma cells achieve the dual goal of stimulating their own growth and invasiveness while killing surrounding cells — an impressive example of the misuse of the communication properties of normal astrocytes.

AIDS-related neuropathology. Astrocytes can become neuronal killers not only through tumour transformation, but also as a result of external triggers — a notable trigger being microglia in the case of AIDS affecting the brain. A subgroup of patients with AIDS develops neurological dysfunctions that lead, in the worst cases, to frank dementia with brain atrophy[132]. As neurons are not infected by HIV, their death must be due to external causes. Microglia are the only cells in the CNS that are productively infected by the virus, and they trigger complex neurotoxic cascades that involve the dysregulation of astrocytes. A crucial site in astrocytes is the chemokine (C-X-C motif) receptor 4 (CXCR4), stimulation of which induces synaptic modulation[133]

through TNFα-controlled, Ca^{2+}-dependent glutamate release[95]. However, the CXCR4 receptor is also a target for HIV (T-tropic strains) and, when it is potently activated by the viral coat protein gp120, a massive localized release of TNFα occurs from both astrocytes and microglia. This amplifies glutamate release from the astrocytes and, eventually, induces excitotoxic neuronal apoptosis[95] (FIG. 5). The evidence that pathological microglia–astrocyte interactions are involved in HIV-induced neuronal death is strengthened by the fact that microglia can also induce an alteration in normal astrocyte-to-neuron chemokine signalling[134].

Alzheimer's disease. Recent work has revealed that healthy adult astrocytes have an important protective role, as they specifically bind, internalize and efficiently degrade amyloid-β (Aβ), the protein that accumulates in the plaques of Alzheimer's disease[135]. In Alzheimer's disease, a defect in this digestive function, together with the abnormal expression of β-secretase, the Aβ-generating enzyme[136], can switch the role of astrocytes to promoters of Aβ accumulation and plaque formation. The precise mechanism by which astrocytes recognize and degrade Aβ is not known, but apolipoprotein E (ApoE)-dependent signalling seems to be crucial[137]. The implication of an ApoE defect is important because this protein, which, in the brain, is produced almost exclusively by astrocytes[12], is a recognized genetic risk factor for Alzheimer's disease. The importance of astrocytic ApoE is also emphasized by its role in the genesis and functionality of synapses[24], which are particularly affected in Alzheimer's disease.

Amyotrophic lateral sclerosis. Amyotrophic lateral sclerosis (ALS) is a fatal disorder that leads to progressive, selective degeneration of upper and lower motor neurons. In a subset of familial cases it is associated with point mutations in the gene that encodes a ubiquitous antioxidant enzyme, Cu/Zn superoxide dismutase (SOD1)[138]. Interestingly, in order for motor neuron pathology to appear, the enzyme needs to be mutated in both neurons and astrocytes[139]. Mice engineered to express mutated SOD1 in only neurons or astrocytes do not develop ALS-like pathology[140–142].

What are the deleterious effects of mutant SOD1 on glial cells and on their dialogue with neurons? Astrocytes that express mutant SOD1 are both activated and damaged. They show focal loss of the glutamate transporter EAAT2 (REF. 143), a defect also observed in patients with sporadic ALS that is probably involved in the pathogenesis of the disease[144]. Reduced uptake might lead to a sequence of events, including increased spill-over of synaptic glutamate, activation of mGluR signalling in astrocytes with further glutamate release[94] and increased generation of nitric oxide[145], an agent that stimulates a pro-apoptotic pathway in motor neurons[146]. Whatever the exact mechanisms, the defective glial partners become unable to keep neurons alive, which causes their death in a vicious cycle of reciprocally altered communication.

Conclusions

During the past few years our understanding of astrocytes and their communication with neighbouring astrocytes, neurons, blood vessel and various glial cells has increased at a faster rate than for any other cell type in the nervous system. In some areas of research, the new information, although inevitably incomplete, already seems to be comprehensive and convincing; in others, however, it is still fragmentary, and might just be the tip of an iceberg. The characterization of astrocytes at the single cell level is still inconclusive. There are strong indications that the cells that we include under the definition of astrocytes are not all the same, but have various morphological, molecular and functional differences. But, no obvious solid criteria have been established for the recognition of their heterogeneity and their classification. In terms of significance, the heterogeneity remains mysterious; for example, the existence of astrocyte subpopulations with different gliotransmitter-secretion properties can only be hypothesized. At the subcellular level, although the existence and autonomous functioning of regions that are specialized for communication with synapses or blood vessels is increasingly recognized, a comprehensive understanding of the structural–functional relationships that govern the cell biology of astrocytes is not yet available.

On the other hand, the morphological finding that each astrocyte occupies a distinct brain territory and might interact stably with several neurons, many nerve fibres and hundreds to thousands of synapses is in agreement with the results of neuronal synchronization studies. These studies have shown that astrocytes not only modulate but also generate signals and bridge pathways and networks, linking cells and structures that are not functionally connected. Astrocytes are peculiar excitable cells, being unable to generate action potentials and yet capable of sending slow feedback and feedforward output responses to their neighbouring cells.

The complex transductive processes by which astrocytes distinguish various types of stimulus and tune their responses accordingly are still poorly understood. A particularly sophisticated example of their functions is their spatial control of $[Ca^{2+}]_i$ changes, which can remain restricted to one or more microdomains, expand to the whole cell or propagate along astrocytic networks, the circuit maps of which remain obscure. What is beginning to emerge, however, is the physiological relevance of the astrocytic interactions through which the activity of neurons can be continuously monitored and regulated, especially at both presynaptic and postsynaptic levels. Another function of physiological relevance is the control of blood flow, which can be continuously adapted to local needs through the induction of local vasoconstriction or vasodilation responses.

The amplitude and complexity of the roles of astrocytes depend considerably on the multiplicity of their released gliotransmitters and co-released factors, such as prostaglandins and TNFα. Through the action of glutamate, ATP, adenosine, D-serine and other factors, astrocytes exert a range of effects, not only on excitatory

but also on inhibitory nerve cells and fibres. In many circuits — including those of the hippocampus, which is notoriously involved in cognitive activity — they might work to fine-tune the balance between excitation and inhibition. The process that has attracted most attention — the release of glutamate — seems to occur primarily through classic exocytosis, which, although more rapid in astrocytes than in other non-neuronal cells, is distinctly slower than at neuronal synapses. By contrast, the exocytotic discharge of other gliotransmitters, and in particular ATP, is still largely mysterious. Moreover, many crucial questions remain unanswered, such as: do astrocytes possess specialized surface regions for SLMV fusion that are similar to the active zones of neuronal synapses? Are these distributed in the same cell at several loci, such as the many perisynaptic expansions? Do the local interactions with neurons and other neighbouring cells occur with high spatial resolution, as they do at synapses, or in a paracrine fashion?

In addition to exocytosis, the release of glutamate, ATP and other gliotransmitters seems to take place through molecular transport mechanisms. Whether, and to what extent, these mechanisms contribute to the regulated release that sustains physiological gliotransmission remains to be seen. Alternatively, their physiological role might be to maintain ambient transmitter concentrations[113,147], and/or they could have more crucial roles in pathological conditions and brain diseases. As a few effectors, such as CX43 hemichannels, $P2X_7$ receptors and cystine/glutamate exchangers, have been identified at the molecular level, the above issues can now be investigated using selective interference techniques (such as cell-specific knockout, small interfering RNA and dominant-negative approaches).

Finally, the correct functioning of astrocytes and their partnership with neighbouring cells seems to be essential for normal brain function. Recognition of this has already changed our perspective on the pathogenesis of several brain diseases. An impressive example is ALS: until recently, it was attributed to primary lesions of motor neurons; however, the pathology has now been shown to depend on alterations in the local interactions between motor neurons, astrocytes and microglial cells. Interestingly, the types of alteration can differ considerably among the various diseases, which reveals opportunities for specific therapeutic interventions. Important progress in this field can, therefore, be expected in the near future, prompting significant developments in our understanding of disease mechanisms and in clinical and therapeutic approaches, which are currently at a primitive stage.

What else? The growing interest of an increasing number of neuroscience laboratories in astrocyte studies probably best illustrates the situation. We have provided a general view of the present knowledge. However, it is easy to predict that in a few years another review will be required to track the ongoing progress in the field: the astrocyte revolution is bound to continue.

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Competing interests statement

The authors declare no competing financial interests.

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